Translated by: Schreiber Translations, Inc.

Country	:	Japan
Document No.	:	2002-20403
Document Type	:	Kokai
Language	:	Japanese
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IPC	:	C 08 B 37/00
		A 61 K 35/80
		A 61 P 25/00
		31/12
		35/00
		37/00
		43/12
Date of Filing	:	July 5, 2000
Publication Date	:	January 23, 2002

Foreign Language Title : Apotoshisu o Yudosuru Shinki Tato oyobi Sono Yoto

English Title : NOVEL POLYSACCHARIDE FOR INDUCING APOPTOSIS AND USE THEREOF

SPECIFICATION

(54) Title of the Invention

Novel Polysaccharide for Inducing Apoptosis and Use Thereof

[Claims]

[Claim 1] A composition, which is characterized by being one or more fractions obtained by fractionating a water extract of seaweeds based on difference in molecular weight and containing polysaccharides that induce an apoptosis selectively for abnormal cells undesirable to a living body.

[Claim 2] The composition according to Claim 1, in which said seaweeds belong to red algae or brown algae.

[Claim 3] The composition according to Claim 1, in which said seaweeds belong to families selected from a group comprising Alaria family, Tangle family, Nemacystus family and Gloiopeltis family.

[Claim 4] The composition according to any of Claims 1 - 3, in which said abnormal cells undesirable to a living body are selected from a group comprising cancer cells, autoreactive cells,

[1]Numbers in the margin indicate pagination in the foreign text.

abnormal nerve cells, viral infection cells and radiation exposed cells.

[Claim 5] Medical compositions, which are medical compositions made by containing a medically efficient amount of the composition according to any of Claims 1 - 4 and is used for prevention and medical treatment of diseases selected from a group comprising cancers, autoimmune diseases, nerve diseases, viral infection diseases and radiation exposure sickness.

[Claim 6] Food products made by containing the composition according to any of Claims 1 - 4.

[Claim 7] Polysaccharides, which have the following properties and induce an apoptosis selectively for abnormal cells undesirable to a living body.

(1) Molecular weight: 5,000 - 100,000

(2) It is constructed in a mole ratio of galactose, fucose and mannose 1 : 0.4±0.1 : 0.3±0.05

(3) It contains glucuronic acid in a mole ratio of 0.6 - 1.0 to 1 galactose.

[Claim 8] The polysaccharide according to Claim 7, which has a molecular weight of 50,000 - 100,000.

[Claim 9] The polysaccharide according to Claim 7 or 8, which originates from seaweeds.

[Claim 10] The polysaccharide according to any of Claims 7 - 9, in which the abnormal cells are selected from a group comprising cancer cells, autoreactive cells, abnormal nerve cells, viral infection cells and radiation exposed cells.

[Claim 11] Medical compositions, which are medical composition with the polysaccharide according to any of Claims 7 - 10 as active constituent and are used for prevention and medical treatment of diseases selected from a group comprising cancers, autoimmune diseases, nerve diseases, viral infection diseases and radiation exposure sickness.

[Claim 12] Food products, which are made by containing the polysaccharide according to any of Claims 1 - 10.

[Detailed Description of the Invention]

[0001]

[Technical Field of the Invention] The present invention relates to a novel polysaccharide which can induce an apoptosis selectively for abnormal cells undesirable to a living body (sometimes simply called ⌈apoptosis inducible polysaccharide⌋), medical compositions with fractions of water extract of seaweeds containing said polysaccharide as active constituents and particularly to medical compositions for prevention and medical treat-ment of diseases (e. g., cancers, colon polyposis, autoimmune diseases such as rheumatism, viral infection

diseases, etc.). The present invention also relates to health food made by containing the above polysaccharide or polysaccharide-containing composi-tions.

[0002]

[Prior Art] The cell death controlled by genes, called apoptosis, plays an important role in fundamental life phenomena such as morphologic changes in ontogeny of higher animals and establishment of network of nerve system, maintenance of homeostasis in endocrine system of mature individual, variety of immune systems and establishment of specificity, etc. It gradually became clear that the apoptosis is not only such a physiological phenomenon, but also is deeply involved in onset of various pathoses, e. g., cancers, viral infections such as AIDs, etc., nerve diseases such as Alzeimer's disease, autoimmune diseases such as chronic arthrorhematism, etc. For diseases involved in such an apoptosis, a search after compounds which induce an apoptosis selectively for their pathogenic cells and have almost no toxicity to normal cells have been prosperously made, and some promising compounds have actually been screened. However, it is present situation that this search has not been connected with the establishment of medical treatment methods and prevention methods based on such compounds so far.

[0003]

[Problems to Be Solved by the invention] Therefore, one purpose of the present invention consists in screening a novel compound having an apoptosis induction ability selective for pathogenic cells such as cancer cells and viral infection cells, etc. and a wide safety region to a living body from natural products and providing medicines for effective and safe prevention and medical treatment of various diseases caused by the above pathogenic cells. Another purpose of the present invention consists in providing health food made by containing said novel compound.

[0004]

[Means for Solving the Problems] In a study for artificially controlling the apoptosis, the inventors advanced a wide search after compounds having an induction activity of apoptosis and almost no toxicity widely from natural products. According to results of study in this field, it becomes clear that the compounds have the apoptosis induction ability for special structures of natural polysaccharides. In the present invention, the inventors paid their attention to seaweeds such as メカブ (mekabu) a part where leaves gather into thick and short folds close to root of wakame seaweed inserted into a rock, see paragraph [0009] translator), tangle, nemacystus and gloiopeltis, etc. and tried to acquire objective polysaccharides having the apoptosis induction ability from these seaweeds. As a result,

they discovered that a substance that induces the apoptosis selectively for pathogenic cells of diseases involved in apoptosis of cancer cells existed in a water extract of seaweeds. Subsequently, the inventors fractionated and purified this substance by ultrafiltration and made a structural analysis and clarified that its active constituent is a novel polysaccharide having a different composition of

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constituent saccharides from conventional well-known saccharides. The inventors further confirmed that said novel polysaccharide even could selectively induce the apoptosis for cancer cells and display an antitumor effect *in vivo*. In a cancer-carrying animal test, thus came to accomplish the present invention.

[0005] Namely, the present invention provides a composition characterized by being one or more fractions obtained by fractionating a water extract of seaweeds based on difference in mole-cular weight and containing a polysaccharide that induces an apoptosis selectively for abnormal cells undesirable to a living body. In a preferable embodiment form, said composition is fractions of MW 5,000 - 100,000, and preferably is fractions of MW 50,000 - 100,000. In such fractions, an apoptosis inducible polysaccharide of the present invention is

(1) constructed in a mole ratio of galactose, fucose and mannose 1 : 0.4±0.1 : 0.3±0.05 and

(2) contains glucuronic acid in a mole ratio of 0.6 - 1.0 to 1 galactose by the above sugar composition.

[0006] The apoptosis inducible polysaccharide and the seaweed originated composition containing said polysaccharide is efficient in prevention and medical treatment caused by abnormal cells undesirable to a living body because they can induce an apoptosis selectively for these cells. Therefore, the present invention provides medical compositions which are made by containing a medically efficient amount of the apoptosis inducible polysaccharide or the seaweed originated composition containing said polysaccharide and used for prevention or medical treatment of diseases such as cancers, autoimmune diseases, nerve diseases, viral infection diseases and radiation exposure sickness.

[0007] Since the apoptosis inducible polysaccharide or the seaweed originated composition containing said polysaccharide are extracts from edible seaweeds and their fractionally purified products or their equivalents, they can be safely utilized for food, and a preventive effect of above diseases can be achieved by oral intake in daily life. Therefore, the present invention also provides food products made by containing the apoptosis inducible polysaccharide or the seaweed originated

composition containing said polysaccharide of the present invention.

[0008]

[Embodiment Form of the Invention] The ⌈apoptosis inducible polysaccharide⌋ of the invention can induce an apoptosis selectively for abnormal cells undesirable to a living body and have the following properties:

(1) Molecular weight: 5,000 - 100,000

(2) It is constructed in a mole ratio of galactose, fucose and mannose 1 : 0.4±0.1 : 0.3±0.05

(3) It is not specially limited if it contains glucuronic acid in a mole ratio of 0.6 - 1.0 to 1 galactose, but preferably has the following properties:

(4) In a gas chromatography under certain conditions (details of conditions will be described in later Actual Example 5), it shows peaks at retention times of 11.9 min and 13.1 min, a substance corresponding to the former is contained in a mole ratio of 200±50 to 1 galactose, and a substance corresponding to the latter is contained in a mole ratio of 10±50 to 1 galactose

(5) A sulfate radical is further contained in a mole ratio of 0.9 - 1.5 to 1 neutral saccharide.

[0009] The ⌈apoptosis inducible polysaccharide⌋ of the invention may be one of any origin, for example, seaweeds,

preferably seaweeds belonging to red algae or brown algae, more preferably seaweeds belonging to Alaria family, Tangle family, Nemacystus family and Gloiopeltis family, even more preferably wakame seaweed, and the most preferably mekabu (a part where leaves gather into thick and short folds close to root of wakame inserted into rock) originated seawood are given.

[0010] The ⌜abnormal cells undesirable to a living body⌟ mean abnormal cells which become pathogens of diseases deeply involved in onset of apoptosis or its inhibition, for example, cancer cells in an cancer, autoreactive cells in an autoimmune disease, viral infection cells in a viral infection disease, abnormal nerve cells in a nerve disease, radiation exposed cells in a radiation exposure sickness, etc. are given. The ⌜abnormal cells undesirable to a living body⌟ preferably are cancer cells, more preferably are mammary cancer cells or glioma cells, etc.

[0011] More preferably, the apoptosis inducible polysaccharide of the present invention has MW 50,000 - 100,000.

[0012] When seaweeds such as wakame (especially mekabu), tangle, nemacystus, gloiopeltis, etc. are taken as raw material, the apoptosis inducible polysaccharide of the present invention can be prepared by the following method. First, dried seaweeds are finely crushed by a crusher, etc., a proper amount of water is added to said crushed matter and fully mixed, then preferably

allowed to stand still at 4 - 10°C for 15 - 25 hr to perform an extraction. After a precipitate is removed by centrifugation, a supernatant is passed through a 100 - 0.45 μm nylon mesh or a filter paper (while reducing pore diameter as necessary) once to several times. The resultant filtrate can be supplied to ultrafiltration to obtain an apoptosis inducible polysaccharide having aforesaid molecular weight and sugar composition, e. g., by fractionating it into fractions of MW over 100,000, 100,000 - 50,000, 50,000 - 5,000 and under 5,000. The resultant fractions can be used and stored as they are an original solution or used by dilution or concentration, or drying or freeze drying.

[0013] The present invention provides a composition which is characterized by being one or more fractions obtained by fractionating a water extract of seaweeds based on difference in molecular weight and containing polysaccharides that induce apotosis selectively for abnormal cells undesirable to a living body. Namely, all the fractionally purified products of water extract of seaweeds obtained by the method as described above, not only fractions of MW 100,000 - 50,000 and 50,000 - 5,000 but

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also fractions of MW over 100,000 and under 5,000 contained in the apoptosis inducible polysaccharide of the present invention have the apoptosis induction ability. The sugar composition of polysaccharides contained in the fraction of MW over 100,000

approximates to the sugar composition of unseparated water extract, thus it is considered to be the most part existing in the seaweed polysaccharide. On the other hand, the sugar composition of polysaccharide contained in the fraction of MW under 5,000 approximates to the sugar composition of polysaccharide contained in the fraction of MW 50,000 - 5,000. Moreover, the ⌈polysaccharide⌋ in the present invention means a saccharic produced by dehydration combination of more than 10 molecules of monosaccharides (including derivatives such as saccharic alcohols or uronic acid). Therefore, the ⌈composition containing apoptosis inducible polysaccharide originated from water extract of seaweeds⌋ of the present invention must be fractions containing at least a polysaccharide including molecular weights of extents defined above.

[0014] The ⌈composition containing apoptosis inducible polysaccharide originated from water extract of seaweeds⌋ of the present invention may originate from any seaweeds, preferably seaweeds belonging to red algae or brown algae, more preferably seaweeds belonging to tangle, nemacystus, gloiopeltis, even more preferably seaweeds originated wameka, and the most preferably seaweeds originated from mekabu.

[0015] The ⌈composition containing apoptosis inducible polysaccharide originated from water extract of seaweeds⌋ of the

present invention preferably originates from fractions containing the apoptosis inducible polysaccharide of the present invention, therefore it preferably originates from fractions of MW 5,000 - 100,000, more preferably MW 50,000 - 100,000.

[0016] The present invention provides medical compositions containing a medically efficient amount of the ⌈apoptosis inducible polysaccharide⌋ or the ⌈composition containing apoptosis inducible polysaccharide originated from water extract of seaweeds⌋ of the present invention. The ⌈apoptosis inducible polysaccharide⌋ or the ⌈composition containing apoptosis inducible polysaccharide originated from water extract of seaweeds⌋ can induce the apoptosis selectively for abnormal cells undesirable to a living body, therefore said medical compositions can be used for prevention and medical treatment of diseases caused by said abnormal cells. As such diseases, for example, cancers such as mammary cancer and glioma, etc., autoimmune diseases such as leukemia, colon polyposis, systemic lupus erythematosus and chronic arthrorhematism etc., inflammatory diseases such as ulcerative colitis and Sjögren's syndrome, etc., nerve diseases such as Huntington's disease, Alzeimer's disease, Parkinson's disease, Down's syndrome, amyotrophic lateral sclerosis, etc., viral infection diseases of HIV, HBV, HCV, adenovirus, poxvirus, herpes virus, papovavirus, etc., exposure sickness caused by

radioactive irradiation, etc. are given. They are medical compositions preferably for prevention and medical treatment of cancers, especially mammary cancer, glioma, etc.

[0017] The ⌈apoptosis inducible polysaccharide⌋ or the ⌈composition containing apoptosis inducible polysaccharide originated from water extract of seaweeds⌋ of the present invention can be properly mixed with necessary ingredients such as medically allowable carriers (e. g., excipient, diluent, etc.) and prepared in such dosage forms as liquid preparation, powder, granule, tablet, capsule, syrup, injection, aerosol, etc., and can be adminstered orally or parenterally.

[0018] As medically allowable carriers, for example, exipients such as sucrose, starch, mannite, sorbit, lactose, glucose, cellulose, talc, calcium phosphate, calcium carbonate, etc.; couplers such as cellulose, methyl cellulose, hydroxypropyl cellulose, polypropylpyrrolidone, sucrose, starch, etc.; disintegrants such as starch, carboxymethyl cellulose, sodium glycol starch, sodium bicarbonate, magnesium sterate, calcium citrate, etc.; lubricants such as magnesium stearate, aerosil, talc, sodium lauryl sulfate, etc.; aromatics such as citric acid, mentol, glycyrrhizin ammonium salt, glycine, orange powder, etc.; preservatives such as sodium benzoate, sodium hydrogen sulfite, methylparaben, propylparaben, etc.; stabilizers such as citric acid, sodium citrate, acetic acid, etc.; suspending

agents such as methyl cellulose, polyvinylpyrrolidone, aluminum stearate, etc.; dispersants such as surfactants, etc.; diluents such as water, physiological saline solution, orange juice, etc.; base waxes such as cacao butter, polyethylene glycol, white lamp oil, etc. are given, but the carriers are not limited thereto.

[0019] Preparations of the present invention are preferably oral or parenteral preparations. Preparations suitable for oral administration are liquid preparations given by dissolving an efficient amount of the 「apoptosis inducible polysaccharide」 or the 「composition containing apoptosis inducible polysaccharide originated from water extract of seaweeds」 in such a diluent as water, physiological saline solution or orange juice; capsule, ｻｯｼｪｰ agent or tablet given by containing an efficient amount of said substances as solid or granule, suspending agents given by suspending an efficient amount of said substances in a suitable dispersion medium; emulsion given by dispersing or emulsifying a solution dissolving an efficient amount of said substances in a suitable dispersion medium, etc.

[0020] As preparations suitable for parenteral administration, aqueous or non-aqueous isotonic aseptic injection, antioxidant, buffer solution, bacteriostat, isotonizing agent, etc. may also be contained in these preparations. Aqueous and non-

aqueous aseptic suspending liquids are given, and suspending agent, solubilizing agent, thickening agent, stabilizer, anti-septic agent, etc. may also contained therein. The preparations of the present invention can be sealed into a container every unit dose or plural doses. An efficient amount of the ⌈apoptosis inducible polysaccharide⌋ or the ⌈composition containing apoptosis inducible polysaccharide originated from water extract of sea-weeds⌋ and a medically allowable carriers can be freeze dried and then stored in a state dissolved or suspended in a suitable aseptic vehicle immediately before use.

[0021] The dose of medical composition of the present inven-tion depends upon the effective amount of apoptosis inducible

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polysaccharide being active constituent, cytotoxicity, kind of disease, the extent of progress of disease, drug receptivity of administration target, body weight, age, etc., for example, in case of oral administration, the dose is commonly 1 - 1,000 mg/kg body weight as amount of polysaccharide being active constituent, and this amount can be administered once a day or by dividing it into several times a day.

[0022] Since the ⌈apoptosis inducible polysaccharide⌋ and the ⌈composition containing apoptosis inducible polysaccharide

originated from water extract of seaweeds」 are compounds which have been safely intaken so far as food or their equivalents, they are substances having an extremely high LD_{50} value and a wide safety region (here, 「equivalents」 are obtained from seaweeds other than food or raw materials other than seaweeds but they are essentailly same substances obtained from edible seaweeds). Therefore, the 「apoptosis inducible polysaccharide」 and the 「composition containing apoptosis inducible polysaccharide originated from water extract of seaweeds」 not only can be used as medicine, but also can be used for food such as health food, etc. separately or with allowable food additives. As allowable food additives, commonly used antiseptic agent, colorant, perfume, stabilizer, etc. are given. They can also be taken as polyfunc-tional nutritive food by mixing with other nutrient such as sugar, protein, lipid, amino acid, vitamins, iron, calcium, magnesium, food fiber, etc.

[0023]

[Actual Examples] The present invention is illustrated in more detail by giving actual examples below, but they are simply illustrations and do not limit the scope of present invention anyway.

[0024] [Actual Example 1] Freeze dried powder of water extract of mekabu

(Preparaton of mekabu polysaccharide)

Mekabu (a dry mekabu produced in Sanriku, Japan) was crushed at 15,000 rpm for 1 min by a blender crusher to give a mekabu powder. Next, 10 L of water was added to 10 g of mekabu powder, fully mixed and then allowed to stand still at 4°C for 24 hr. A precipitate was removed by high-speed centrifugation (3,000 rpm, 5 min) and the supernatant was recovered. The solution passed through a 70 μm nylon mesh and a 45 μm nylon mesh once for each. It further passed through a Whatman GD/X (0.45 μm) filter once, a fraction of MW under 100,000 were collected by ultrafiltration method and made into a powder (mekabu polysaccharide).

[0025] [Actual Example 2] Apoptosis induction activity of mekabu polysaccharide to culture strains of cancer cells

Culture strains of rat mammary cancer cells induced by 9,10-dimethylbenzene anthracene (DMBA) were sowed in a 60 mm Petri's dish so that they became $5x10^5$ cells/mL, cultured at 37°C in 5% CO_2-95% atmosphere overnight in a CO_2-incubator, then the above mekabu polysaccharide was added to a culture medium at various concentrations to further carry out the culture. Cells floating in the culture medium were recovered, and residual cells adhering to the surface of Petri's dish were recovered by trypsin/EDTA medical treatment and combined. The recovered cells were washed with PBS(-), then treated at 37°C for 40 min with 40

μL of RNase (1 mg/mL)/sample. The cells were washed with PBS(-) twice and dyed for 30 min on shielded ice with 100 μL of propidium iodide/sample. The cells passed through a 70 μm nylon mesh, and the DNA fragmentation of cells were quantitatively estimated by a flow cytometer (Bechman Coulter E picsXL-2). The result is shown in Table 1.

[0026]

[Table 1]

	Apoptosis Induction Activity (%)			
Treatment Time (hr)	Concentration (mg/mL)			
	0.03	0.12	0.48	0.96
0	4	5	3	4
24	6	6	7	8
48	9	24	37	45
72	11	36	51	54
96	28	47	62	63

[0027] It was known that the mekabu polysaccharide has an ability for inducing apoptosis to the strains of rat mammary cancer cells depending on concentration and time.

[0028] [Actual Example 3] Cell selectivity of the apoptosis induction abiliity of mekabu polysaccharide

The apoptosis induction abiliity of mekabu polysaccharide (0.48 mg/mL) to two kinds of culture strains of human mammary cancer cells (T47D and MDA-MB231) and strains of normal mammary glands (MCF-10A) was similarly examined as Actual Example 2. The result is shown in Table 2.

[0029]

[Table 2]

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	Apoptosis Induction Activity (%)		
Treatment Time (hr)	Cell strains		
	T47D	MDA-MB231	MCF-10A
0	8	4	4
24	18	35	7
48	27	48	6
72	36	51	4
96	42	52	5

[0030] It was known that the mekabu polysaccharide induces apoptosis to mammary cancer cells but does not exhibit the apoptosis induction ability for normal mammary gland cells.

[0031] [Actual Example 4] Comparison of apoptosis induction abilities of mekabu polysaccharide and 5-fluorouracil (5-FU)

The apoptosis induction abilities of mekabu polysaccharide (0.48 mg/mL) and 5-FU (2 and 20 μg/mL) most frequently used for medical treatment of human mammary cancer to culture strains of human mammary cancer cells (T47D) were compared and studied. The result is shown in Table 3.

[0032]

[Table 3]

	Apoptosis Induction Activity (%)		
Treatment Time (hr)	Mekabu Poly-saccha-ride	5-FU	
	0.48 (mg/mL)	2 (μg/mL)	20 (μg/mL)
0	5	5	4
24	9	8	6
48	26	13	22
72	48	28	34
96	58	37	54

[0033] They were compared in respective optimum concentration ranges, consequently it was known that the mekabu polysaccharide has a slightly stronger apoptosis ability than 5 -FU.

5-FU has strong cytotoxicity and essentially cannot be used at a higher concentration.

[0034] [Actual Example 5] Fractionation by ultrafiltration of mekabu polysaccharide and sugar composition analysis of fractionally purified products

A mekabu polysaccharide solution was taken as (I) of MW over 100,000, (II) of MW 100,000 - 50,000, (III) of MW 50,000 - 5,000 and (IV) of MW under 5,000 by ultrafiltration method. A comparison analysis of saccharide compositions of the fractionally purified products was made by using unseparated mekabu polysaccharide and fucoidan extracted from said mekabu as controls. Each fraction crystallized with ethanol was dissolved in reference water and taken as sample. The analysis of constituent saccharides was quantified by gas chromatography according to Reinhold's method [Reinhold, V.N., *Methods in Enzymology*, **25B**, 244 - 249 (1972)]. Chromatographic conditions are as follows.

[0035] Chromatograph: Shimazu GC14A column: capillary column (Shimazu CBJ15, 0.32 mm x 30 mm)

Heating conditions: heating from initial temperature 180°C to 250°C at 5°C/min

Carrier gas: nitrogen gas

Detector: hydrogen flame ionization detector

Quantification: Shimazu integrator C-86 chromatopack

[0036] The neutral saccharides were quantified with galactose as standard according to a phenol sulfuric acid method {Dubois, M., Gilles, K.A. et al, *Anal. Chem.* **28**, 350 (1956)]. The sulfate radical was quantified by a rhodizonic acid method [Terho, T.T. and Hartiala, K. *Anal. Biochem.*, **41**, 471 (1971)] with chondroitin sulfate C as standard substance. The results are shown in Tables 4 and 5.

[0037]

[Table 4]

Sugar	Fraction					
	Unsepa-rated	I	II	III	IV	Mekabu Fucoidan
Fucose	0.99	1.1	0.47	0.32	0.32	1.4
Galactose[a)]	1.0	1.0	1.0	1.0	1.0	1.0
Mannose	0.060	0.060	0.35	0.33	0.28	0.041
Glucuronic acid	0.11	0.098	0.92	0.61	0.73	n.d.[b)]
X[c)]	5.7	4.3	200	200	180	n.d.
Y[c)]	1.1	0.60	14	8.1	11	n.d.

a) Galactose was taken as 1 to calculate the mole ratio of each constituent.
b) Not detected.
c) Unidentified peak.

[0038]

[Table 5]

Unit:

mg/mL

	Fraction					
	Unsepa-rated	I	II	III	IV	Mekabu Fucoidan
Neutral sugars	2.1(1.0)[a)b)]	3.1(1.0)	0.12(1.0)	0.13(1.0)	0.10(1.0)	3.3(1.0)
Sulfuric acid[c)]	1.0(1.1)	1.3(0.93)	0.066(1.3)	0.054(0.96)	0.068(1.5)	2.9(2.0)

a) The molecular weight was taken as 180 to calculate the mole number.
b) The inside of parenthesis indicates the mole ratio. However, it was calcu-lated by taking neutral sugar as 1.0.
c) Calculated by taking the weight of sulfuric acid of chrondroitin sulfate as standard.

[0039] Fucose, galactose and mannose are contained as neutral sugars. If galactose was taken as standard (1.0) when unseparated, the ratio of fucose to mannose is 1:0.6, but all the ratios in the fractions II, III, IV are 0.4±0.1 : 0.3±0.05. The mole ratio of glucuronic acid to galactose is 0.1 when unseparated, while the content becomes as high as 1 - 0.6 in all the fractions II, III, IV. Unidentified peaks X, Y with high content existed in the fractions II, III, IV. From retention times in chromatography (X: 11.9 min, Y: 13.1 min), there is a high possibility that they are saccharic alcohols like mannitol

and inositol. Therefore, the sugar composition of Honta sugar is different from mekabu fucoidan.

[0040] On the other hand, the content of sulfate (radical) which is a characteristic of fucoidan is as very low as about 1/50 of fucoidan in all the fractions II, III, IV, and this is still characteristically different from mekabu fucoidan. The mole ratio of sulfate radical to neutral sugars of mekabu polysaccharide is about 1 (0.9 - 1.5) when unseparated and in all the fractions II, III, IV, by contrast, it is about 2 in mekabu fucoidan.

[0041] [Actual Example 6] Apoptosis induction activity of mekabu polysaccharide

The apoptosis induction capacity for culture strains of human mammary cancer cells (T47D, MDA-MB231, MCF-7) was estimated by same method as Actual Example 2 with concentration of sample as 0.48 mg/mL and medical treatment time as 96 hr. The result is shown in Fig. 1. The apoptosis induction capacity was also observed in all the fractions, but a higher apoptosis induction activity was recovered in the fractions of MW under 100,000 than unseparated one, particularly, the fraction II of mW 100,000 - 50,000 showed a high effect about 1.5 times as much as that of unseparated one.

[0042] [Actual Example 7] Carcinogenesis inhibitory effect of mekabu polysaccharide *in vivo*

DMBA dissolved in olive oil was endogastrically administered to 48 eight-week old Sprague-Danley series rats at 20 mg/individual/1 mL olive oil, and all the rats were arbitrarily divided into four groups (n = 12) after one week. Mekabu polysaccharide was put into a 400 mL water feed bottle and orally intaken (administered) in a free amount with water for an original solution (6 mg/mL) intake group (M-A), a doubly diluted solution (3 mg/mL) intake group (M-B), a four-time diluted solution (1.5 mg/mL) intake group (M-C) and a control group. The water feed bottle was washed every two days, exchanged, and its intake amount every time was measured, and the intake amount every week was calculated. The body weight change of rats, the number of tumors where mammary cancer generated by palpation and all tumor diameter were measured for 32 weeks after the administration of DMBA. At the end of experiment of 32th week, collection of blood samples, extraction of all tumors of mammary glands and extraction of various internal organs were carried out, and the weight and diameter of tumors of mammary glands were measured.

[0043]

The results are shown in Figs. 2 - 6. No differences in body weight change, intake amount were found and the safety of long-term intake of mekabu polysaccharide was suggested in all groups. The control groups took an occurrence rate of mammary

cancer of 100% in the 13th week after the administration of DMBA, but a significantly high carcinogenesis inhibitory effect was found in all of the M-A group (100% in the 23th week), M-B group (83.3% in the 32th week) and M-C group (25% in the 32th week) as compared with the control group (Fig. 2). In a comparison of numbers of occurrence of mammary gland tumors, an inhibitory effect on numbers of occurrence of mammary gland tumors was found in the control group (average 7.2 in a comparison of the 32th week at the end of experiment), while the numbers are 2.2, 1.1, 0.3 in M-A, M-B, M-C groups, respectively (Fig. 3). From the above results, it was clarified that mekabu polysaccharide has an effect of inhibiting the occurrence of mammary cancer and proliferation of mammary cancer in the DMBA-induced rat experimental system.

[0044] [Actual Example 8] Preparation Example

The fraction II (MW 100,000 - 50,000) of water extract of mekabu obtained by Actual Example 5 was dried and made into a powder, then 200 g of lactose was added to 1,000 g of said dry powder and mixed with it, and 15 - 20% of water was added and kneaded. This kneaded matter was applied to a pelletizer to granulate it, then dried and tabletted.

[0045] [Actual Example 9] Food Preparation Example (1)

Preparation of mekabu

The fraction II (MW 100,000 - 50,000) of water extract of mekabu obtained by Actual Example 5 was dried and became a powder, then a mekabu candy having a composition described in Table 6 was prepared by ordinary method.

[0046]

[Table 6]

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Material	Weight
Granulated sugar	447 g
Thick malt syrup	430 g
Water	50 g
Dry powder of fraction II of water mekabu extract	40 g 2 g
Table salt	1 g
Tangle extract	

[0047] [Actual Example 10] Food Preparation Example (2)

Preparation of mekabu nutritive capsule

A water mekabu extract obtained in the same way as Actual Example 1 (however, the ultrafiltration was not performed) was dried and made into a powder, then a capsule having a composition described in Table 7 (a tear-drop shaped soft capsule by a gelatin formula described in Table 8) was prepared.

[0048]

[Table 7]

Raw Material	Mixing Weight
Sodium vitamin C	5 mg
Vitamin B1	0.3 mg
Vitamin B2	0.3 mg
Vitamin E (1000 IU/g)	5 mg
β-Carotene	3 mg
Sesame oil	30 mg
Royal jelly	5 mg
Oligosaccharide	5 mg
Dry powder of water mekabu extract	100 mg

[0049]

[Table 8]

Raw Material	Mixing Ratio (part by weight)
Gelatin	100
Glycerin	35
Purified water	70

[0050]

[Effects of the Invention] The apoptosis inducible polysaccharide and the composition containing apoptosis inducible polysaccharide originated from water extract of seaweeds of the present invention can induce an apoptosis selectively for abnormal cells undesirable to a living body, therefore they are efficient

for prevention and medical treatment of diseases caused by these cells. Moreover, said apoptosis inducible polysaccharide and said composition containing apoptosis inducible polysaccharide origi-nated from water extract of seaweeds have a very wide safety region and can be orally intaken in daily life, therefore they are also useful as material of food such as health food useful for the prevention of above diseases.

[Brief Description of the Drawings]

[Fig. 1] A chart showing the apoptosis induction activity of fractionally purified products an water extract of mekabu versus various culture strains of human mammary cancer cells.

[Fig. 2] A graph showing the change of occurrence rate of mammary cancer in rat groups administered with various concentrations of mekabu polysaccharide and non-administered groups with time.

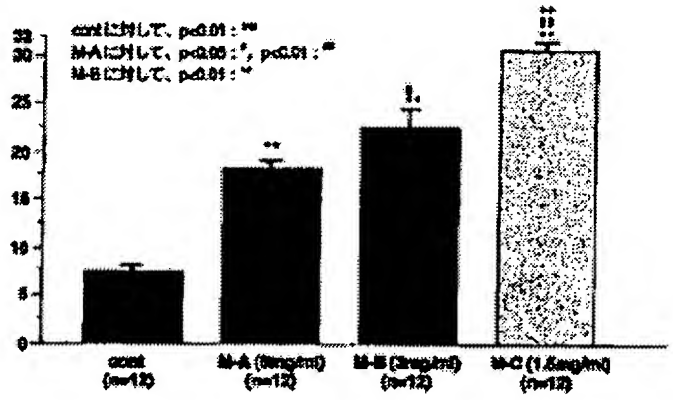
[Fig. 3] A graph showing the time-elapsed change of average number of tumors of mammary cancer per rat in rat groups administered with various concentrations of mekabu polysaccharide and a non-administered groups.

[Fig. 4] A chart showing the average period till onset of mammary cancer in rat groups administered with various concentrations of mekabu polysaccharide and a non-administered group.

[Fig. 5] A chart showing the average weight of tumors during the 32th week in rat groups administered with various concentrations of mekabu polysaccharide and a non-administered group.

[Fig. 6] A chart showing the average surface area of tumors during the 32th week in rat groups administered with various concentrations of mekabu polysaccharide and a non-administered group.

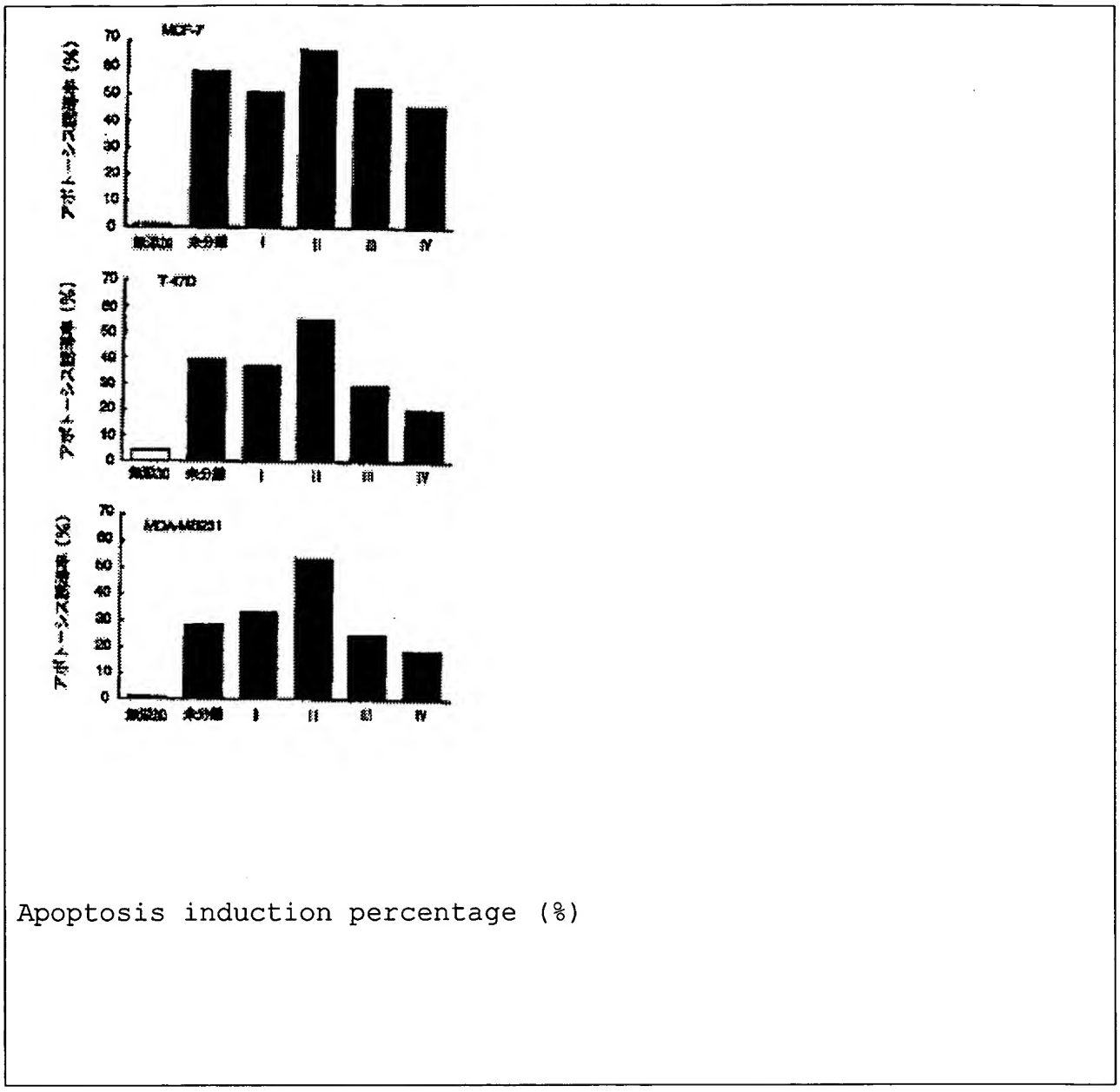
[Fig. 4]



P<0.01 for cont
P<0.05, p<0.01 for M-A
P<0.01 for M-B

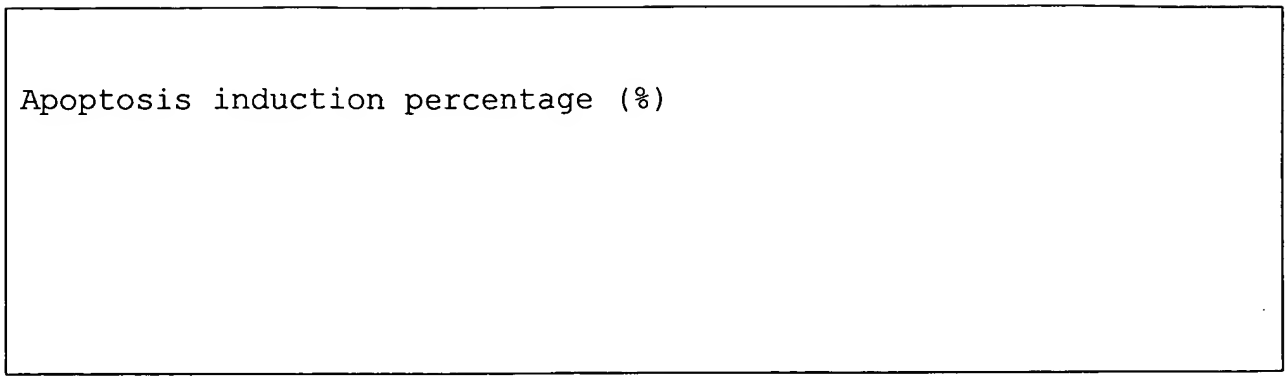
Period till onset of mammary cancer

[Fig. 1]



Apoptosis induction percentage (%)

No addition Unseparated



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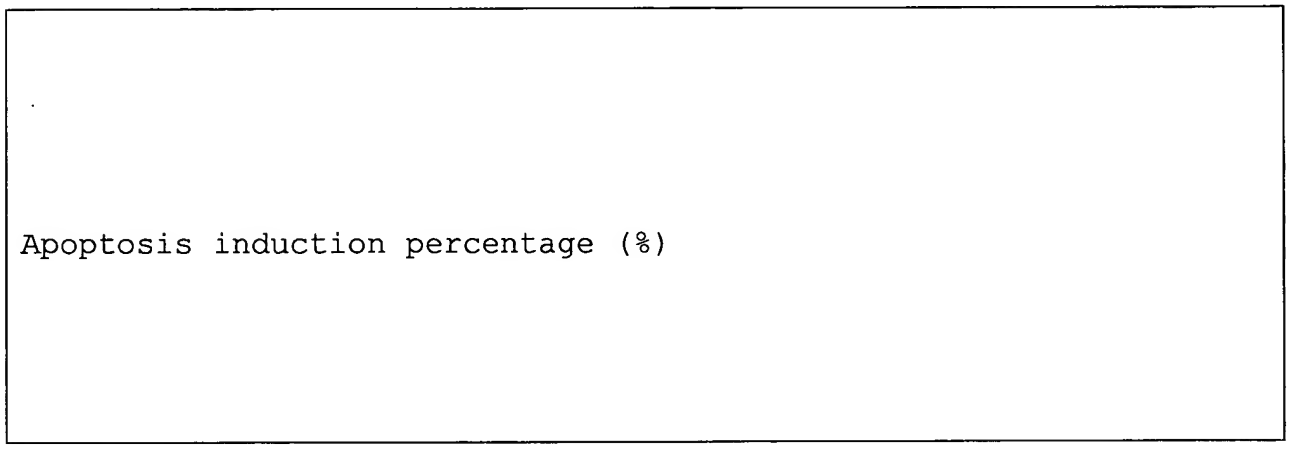
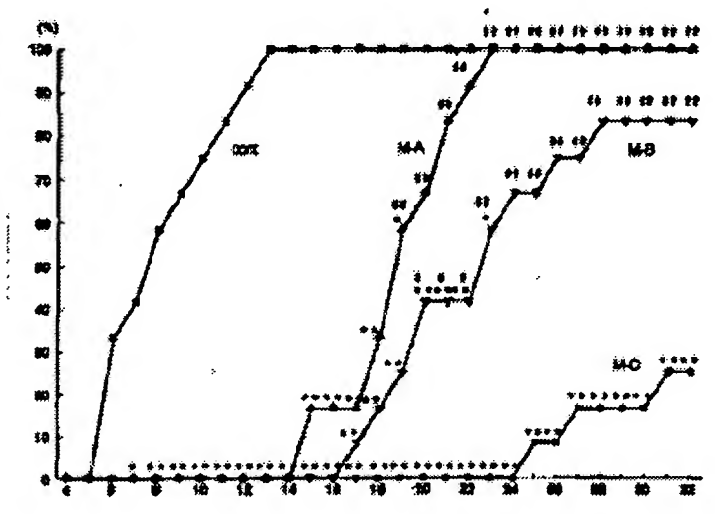


No addition Unseparated

[Fig. 2]



Occurrence rate of mammary cancer

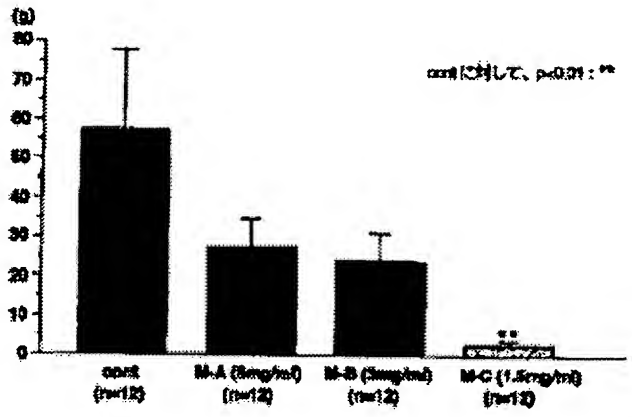
Weeks after administration of DMBA

P<0.05, p<0.01 for cont

P<0.05, p<0.01 for M-C

P<0.05 for M-B

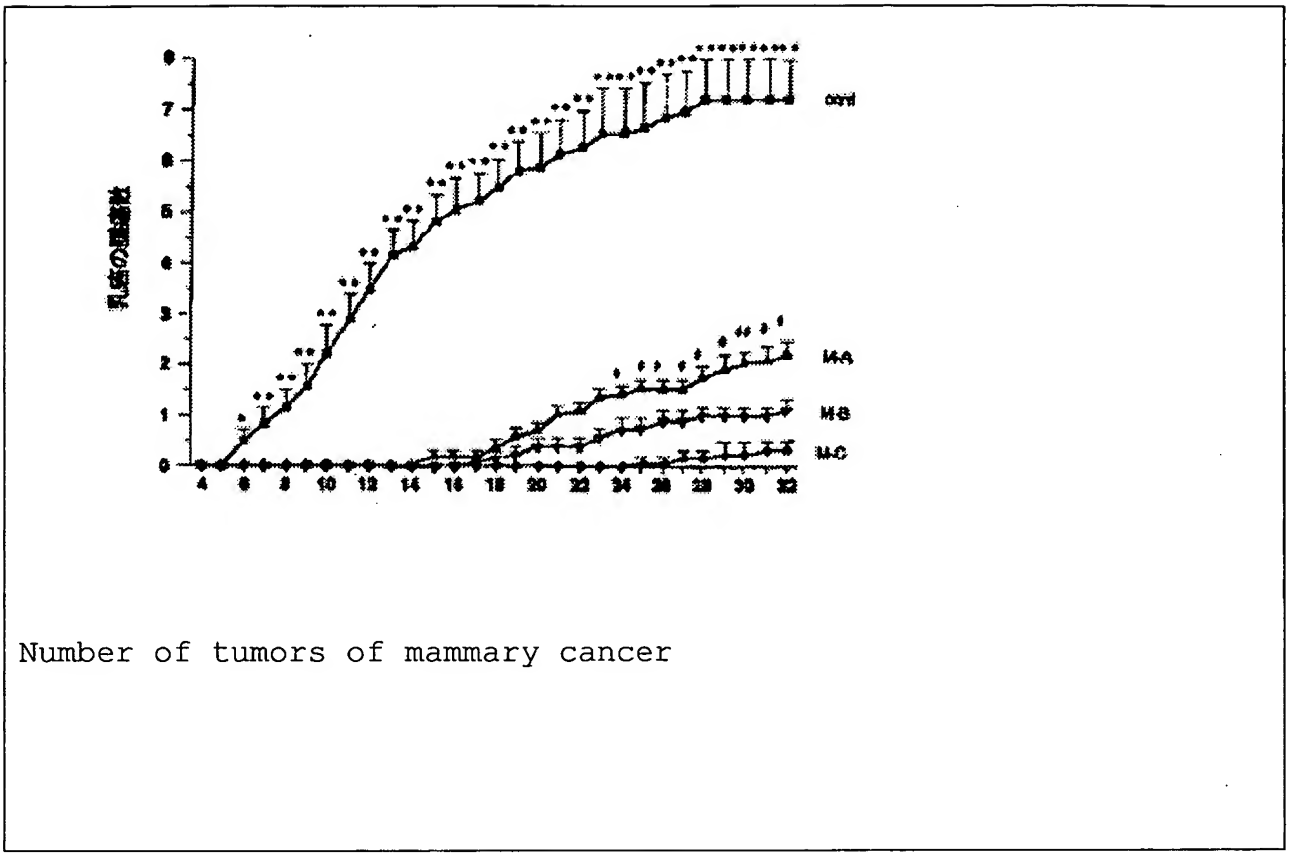
[Fig. 5]



P<0.01 for cont

Total weight of tumors

[Fig. 3]



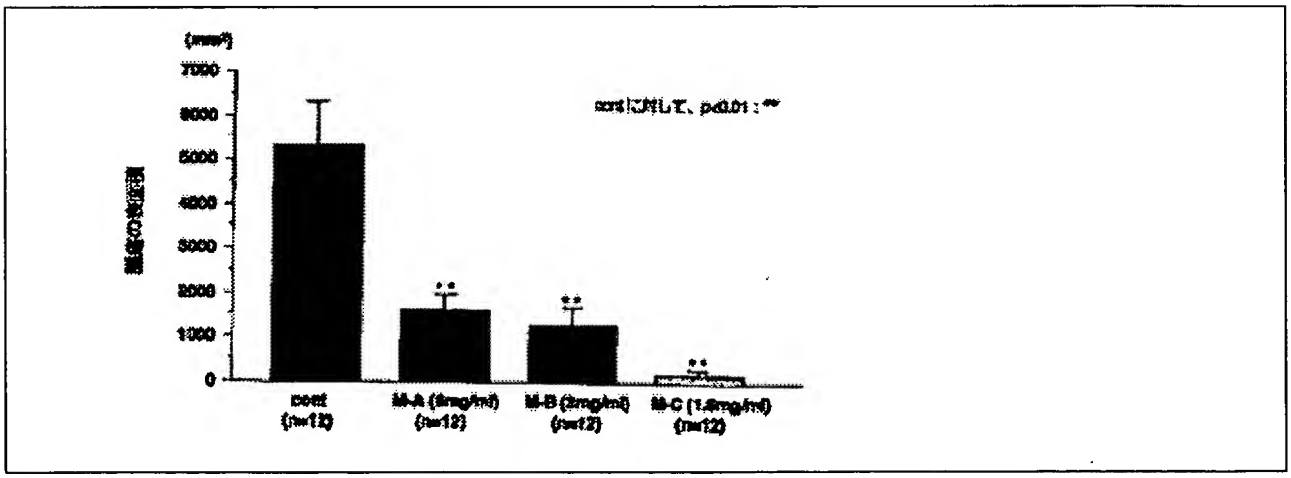
Number of tumors of mammary cancer

Weeks after administration of DMBA

P<0.05, p<0.01 for M-A, M-B and M-C

P<0.05, pz,0.01 for M-C

[Fig. 6]



P<0.01 for cont

Surface area of tumors